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Application of seminested PCR for detection of larval stages of *Spirocerca lupi* in garden lizards (*Calotes versicolor*)

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ABSTRACT

The study reveals the usefulness of semi-nested PCR as a tool to identify larval stages of *Spirocerca lupi* in garden lizards (*Calotes versicolor*), the paratenic host for the parasite. Creamy white cysts present in the superficial muscles of seven out of thirteen garden lizards on the veterinary college campus, Pookode, were recovered and observed for the presence of live larvae. DNA isolated from these cysts and *S. lupi* worms recovered during postmortem examination of an infected dog were used for semi-nested PCR for amplifying an internal region of the *S. lupi* Cox-I gene, producing a specific 400 bp fragment. Thus the semi-nested PCR may be utilized for specific detection of larval stages of *S. lupi* in a paratenic host.

Key words: canine spirocercosis, *Spirocerca lupi*, garden lizards, semi-nested PCR

Introduction

Spirocerca lupi is a spirurid worm, which occurs on the walls of the oesophagus, stomach, aorta and rarely in other organs of dogs, foxes, jackals and wild felidae (SOULSBY, 1982). Canine spirocercosis is characterized by variable clinical signs,

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such as: regurgitation, depression, weight loss, anaemia, melena, vertebral spondylitis, hypertrophic osteopathy, dyspnoea, aspiration pneumonia, pyothorax and neurological disorders and other abnormalities due to aberrant spinal migration (MAZAKI-TOVI et al., 2002).

Garden lizards act as the paratenic host for this parasite. Dogs acquire this infection by ingestion of garden lizards. Available reports on the prevalence rate of spirocercosis and *S. lupi* larvae in garden lizards are very old and scanty (CHANDRASEKHARON et al., 1958; ANANTARAMAN and SEN, 1966). There is no recent study on the prevalence of spirocercosis in dogs or *S. lupi* in garden lizards from southern India. Moreover, the specific detection of larval stages in garden lizards is possible only by observation of larvae or by experimental infection using a naive canine host. These procedures are difficult and molecular methods are promising alternative tools for easy and early characterization of these larvae. This study deals with the application of a semi-nested PCR (CASIRAGHI et al., 2001) for specific detection of larval stages of *S. lupi* in garden lizards.

Materials and methods

Thirteen garden lizards were collected from the college campus and sacrificed using anaesthetic (chloroform) over dosage. The skin was incised to expose the superficial muscle for visual examination of creamy white cysts. The cysts present, if any, were recovered and observed under light microscope for the presence of live larvae.

These cysts were used for isolation of DNA based on SAMBROOK and RUSSEL (2001). DNA isolated from *S. lupi* worms recovered during postmortem examination of an infected dog was used as positive control for the PCR.

The semi nested PCR, amplifying an internal region of the *S. lupi* Cox-I gene, was standardized. In the first round, a 689 bp long region of the Cox-I gene was amplified using the primers NTF (5'-TGA TTG GTG GTT TTG GTA A-3') and NTR (5'-ATA AGT ACG AGT ATC AAT ATC-3') as previously described for Spirurida (CASIRAGHI et al., 2001). For the second round, the forward primer SIIT (5'-TGA CTT TGG ATC AGA TAA G-3') was used, along with NTR (TRAVERSA et al., 2008) to achieve specific amplification of a 400 bp product. For a single reaction (25 µL), 0.5 µL of each primer (100 pmol) and 2 µL of template DNA were used. Amplification reactions were performed in a thermal cycler with a heated lid (Eppendorf, Germany) using the following the cycling protocol: 12 minutes at 94 °C, 40 cycles at 94 °C for 1 minute (first round), 45seconds (second round), 54 °C (first round) or 60 °C (second round) for 1 minute and 72 °C for 1 minute followed by final extension at 72 °C for 10 minutes. The PCR products were electrophoresed and visualized by ethidium bromide under UV trans-illumination (Alpha Digidoc, USA).

Results

Larvae were found in the lizards mainly in the thoracic muscles in an encapsulated form. Out of the 13 garden lizards examined, 7 (53.8 %) showed creamy white cysts (Fig. 1), less than 1 mm in diameter containing larvae (Fig. 2) of *S. lupi*. The maximum number of larvae recovered from a single garden lizard was 21.



Fig. 1. Garden lizard showing the cyst in thoracic muscles



Fig. 2. Cyst showing the *S. lupi* larvae inside

After completion of two rounds of amplifications by the semi nested PCR, the expected 400 bp fragment was observed. DNA from both the *S. lupi* worm as well as the cysts revealed similar sized products, clearly confirming the larval stage of the parasite in the cysts of the paratenic host (Fig. 3).

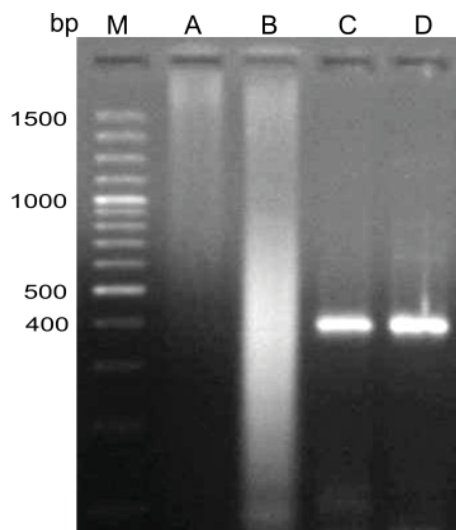


Fig. 3. *S. lupi* specific semi-nested PCR. M - Molecular weight marker (100 bp ladder plus). A, B - products of first round PCR from worm and cyst. C, D - products of second round PCR from worm and cyst

Discussion

The most important factors affecting the prevalence rate of spirocercosis in dogs are proximity to intermediate and paratenic (transport) hosts and the population density of infected and intermediate hosts (VAN DER MERWE et al., 2008). The infective larvae are capable of utilizing a great variety of paratenic hosts, including poultry, wild birds, lizards, rodents, hedgehogs and rabbits (FOX et al., 1988). In previous reports, the prevalence rate of spirocercosis in India varied from 23 % in fecal surveys to 23.5 % from necropsy results (CHANDRASEKHARON et al., 1958). Later, ANANTARAMAN and SEN (1966) reported a prevalence of 88.5 % for *S. lupi* larvae in garden lizards in Chennai. The present study report a prevalence of 53.8 % for the larvae in the paratenic host.

Previously, this PCR technique (CASIRAGHI et al., 2001) was employed for detection of the parasite from faeces. Its use for detection of parasite DNA from the cysts of a paratenic host has not been previously reported. This study is the first confirmed report of paratenic status of garden lizards for transmission of *S. lupi* infection from the state of Kerala, Southern India.

The reason for death in at least a few suspected cases of canine rabies brought to the College of Veterinary and Animal Sciences, Pookode, for post-mortem examination, was attributed to oesophageal tumors caused by *S. lupi*. These animals showed anorexia,

difficulty in swallowing, vomiting and nervous signs prior to death. The neurological disorders due to migration of the larvae and occlusion of the food passage due to the presence of tumors in the oesophagus, resulting in choking, may mimic rabies-like symptoms in canines. Since the mode of transmission of *S. lupi* to dogs is by ingestion of a paratenic host, the present finding of the high prevalence of infection in garden lizards is of utmost importance, since the canine pet population is trend in this state.

The present study utilized a previously standardized PCR technique (CASIRAGHI et al., 2001) for detection of larvae of *S. lupi* from its paratenic host for the first time. Moreover, the results of the study revealed a high prevalence of the larvae in the paratenic host, indirectly indicating the reason for the high prevalence of the disease in dogs the same area.

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SAŽETAK

Istraživanje je provedeno u svrhu provjere učinkovitosti primjene poluugniježđene lančane reakcije polimerazom za identifikaciju ličinaka oblića *S. lupi* u vrtnog guštera (*Calotes versicolor*). Žućkasto bijele ciste bile su izdvojene iz površinskih mišića sedam od 13 vrtnih guštera ulovljenih na području kampusa veterinarskog koledža Pookovode i pretražene na prisutnost živih ličinaka. DNA izdvojena iz tih cista i DNA iz oblića *S. lupi* izdvojenih tijekom razudbe invadiranoga psa rabljene su u poluugniježđenoj lančanoj reakciji polimerazom za umnožavanje unutarnjeg područja gena Cox-I i prepoznavanje specifičnog fragmenta od 400 bp. Zaključeno je da se poluugniježđena PCR može upotrijebiti za dokaz ličinaka oblića *S. lupi* u paratениčnih domaćina.

Ključne riječi: spirocerczoa pasa, *Spirocerca lupi*, vrtni gušteri, poluugniježđena lančana reakcija polimerazom
